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TITLE: Combinational Targeting of Porstate Carcinoma Cells and Tumors Associated Pericytes with Antibody Based Immunotherapy and Metronomic Chemotherapy

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14. ABSTRACT

The hybridoma secreting the HMW-MAA-specific mAb 225.28 which is used for immuno prevention of prostate carcinoma and the hybridoma secreting the isotype matched mAb F3C25 have been tested for activity. Ascitis has been prepared and monoclonal antibodies have been purified and monitored for purity and activity. The colony of TRAMP mice has been expanded to test the efficacy of mAb 225.28 plus cyclophosphamide metronomic therapy in the inhibition of progression of prostate cancer. Sixty-four TRAMP mice have been enrolled in the combinatorial treatment schedule. Animals are being screened 2 times a week for palpable tumors.

15. SUBJECT TERMS
combinatorial therapy, metronomic chemotherapy, monoclonal antibody

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Combinatorial targeting of prostate cancer cells and tumor associated pericytes with antibody-based immunotherapy and metronomic chemotherapy.

PROGRESS REPORT

INTRODUCTION

The lack of efficacy of conventional therapies in prostate cancer has stimulated interest in developing and implementing novel therapeutic strategies. Among them is immunotherapy. To immunize the negative impact of escape mechanisms on the outcome of immunotherapy, the present proposal aims at showing that the efficacy of immunotherapy of prostate cancer can be enhanced by targeting not only cancer cells, but also activated pericytes in the tumor microenvironment with a combinatorial immunotherapy. The latter includes AN-2-specific monoclonal antibody and continuous administration of low dose cyclophosphamide, i.e. metronomic chemotherapy.

BODY

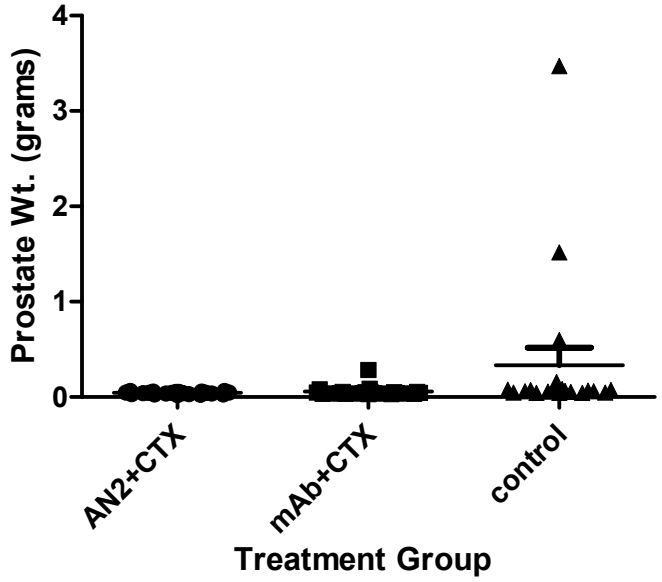
Long Term Treatment to Palpable Tumor

The first replicate of the long term treatment with time to palpable tumor as the endpoint has been completed. We treated 64 TRAMP mice with the combinatorial treatment schedule. TRAMP male mice were started on therapy at 10 weeks-of-age and were maintained on treatment until palpable tumors formed. Animals were assigned to one of three combinatorial treatments. Cohort 1 (n=21) received AN2 mAb treatment twice weekly ip (100 µg/mouse/injection) in combination with metronomic chemotherapy of cyclophosphamide in the drinking water (~10mg/kg/d). Cohort 2 (n=21) received isotype control mAb (100 µg/mouse/injection) in combination with cyclophosphamide (~10mg/kg/d). Cohort 3 (n=22) received no mAb treatment or metronomic chemotherapy. Animals were screened 3 times a week (MWF) for palpable tumors. Antibody injections were given on Tuesdays and Fridays. The results were ambiguous. It appeared upon necropsy that with combination treatment of MCC and AN2 mAb the disease shifted with a decrease in prostatic disease and an increase in seminal vesicle disease. There were animals that needed to be euthanized without palpable disease. The increase in the variability in the presentation of the disease makes the data uninterpretable. Instead of pursuing this study design where the treatment appeared to be altering course and phenotype of the disease, we decided to alter the study to a short term combinational therapy as described below. Briefly animals were treated starting at 12 weeks-of-age, a time at which TRAMP animals have cancer. Animals were treated for 6 weeks (18 weeks-of-age) at which time animals were euthanized and necropsied. The timepoint of 18 weeks-of-age was chosen because TRAMP animals at the age have progressive disease, but minimal numbers of untreated animals are expected to have large palpable tumors. We expect that effective therapeutic approaches will decrease prostate

size and tumor grade. The shorter treatment time frame minimizes the likelihood that selective pressure of the treatment will alter the course of the disease.

Treatment from 12-18 Weeks of Age

The first replicate of the short term treatment has been completed. We have treated 70 TRAMP mice with the combinatorial treatment schedule. TRAMP male mice were started on therapy at 12 weeks-of-age and were maintained on treatment until 18 weeks of age. Animals were assigned to one of three combinatorial treatments. Cohort 1 (n=21) received AN2 mAb treatment twice weekly ip (100 µg/mouse/injection) in combination with metronomic chemotherapy of cyclophosphamide in the drinking water (~10mg/kg/d). Cohort 2 (n=21) received isotype control mAb (100 µg/mouse/injection) in combination with cyclophosphamide (~10mg/kg/d). Cohort 3 (n=32) received no mAb treatment or metronomic chemotherapy. Antibody injections were given on Tuesdays and Fridays. Animals were monitored for signs of toxicity throughout the treatment. At the end of the treatment period animals were euthanized and data collected. At the time of necropsy the prostate was dissected into individual lobes and the following were collected: animal weight, UG weight, combined dissected prostate weight, blood, gross observations for distant metastasis. The following tissues were collected and processed for histology in 9 chamber cassettes: dorsal prostate, lateral prostate, ventral prostate, anterior prostate, seminal vesicles, pelvic lymph nodes, liver, lung and kidney. Based on gross observation at the time of necropsy, no signs of metastasis were observed in cohort 1, 1 case of metastasis was observed in cohort 2 and 2 cases of metastasis in cohort 3. Preliminary analysis of prostate weight indicates cohort 1 and cohort 2 were different from no treatment control (cohort 3), but were not different from each other. All of the tissue samples have been processed and sections cut. One slide from each block has been stained with hemotoxylin and eosin. Currently we are scoring all of the slides to determine micrometastatic incidence and tumor grade.



Cohort 1 (MCC+AN2, n=27) is receiving AN2 mAb treatment twice weekly ip (100 µg/mouse/injection) in combination with metronomic chemotherapy of cyclophosphamide (MCC) in the drinking water (~10mg/kg/d). Cohort 2 (MCC+mAb, n=23) is receiving isotype control mAb (100 µg/mouse/injection) in combination with cyclophosphamide (~10mg/kg/d). Cohort 3 (n=20) is receiving no mAb treatment or metronomic chemotherapy.

KEY RESEARCH ACCOMPLISHMENT

- Analysis of twenty-one TRAMP male mice treated with AN2-specific monoclonal antibodies and with metronomic chemotherapy has detected no side effects. Therefore the combinatorial immunotherapy we propose is safe.
- Macroscopic analysis of the organs harvested from mice treated with the combinatorial immunotherapy has detected no metastases. On the other hand metastases were detected in the control groups.

REPORTABLE OUTCOMES

Background information has been obtained to optimize the antibody-based immunotherapeutic strategy for the treatment of prostate carcinoma.

CONCLUSION

The data obtained thus far indicate that administration of AN2-specific monoclonal antibody in combination with metronomic chemotherapy to TRAMP mice does not cause side effects. In addition the data we have obtained suggest that the combinatorial immunotherapeutic strategy we have developed may have a beneficial effect on the clinical course of prostate carcinoma.